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(54) Title: COMBINATION MENINGITIDIS B/C VACCINES		
(57) Abstract The present invention is directed to a combination vaccine for <i>Neisseria meningitidis</i> comprising outer membrane proteins from serogroup B and oligosaccharides from serogroup C, and its use for the prevention or treatment of disease.		

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COMBINATION MENINGITIDIS B/C VACCINES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. provisional application Serial
5 Numbers 60/087,351 filed May 29, 1998 and 60/106,446 filed October 30, 1998, each of
which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to combination immunogenic compositions and
10 vaccines for *Neisseria meningitidis* B and C and to methods of inducing an immune response
by administering the same.

BACKGROUND OF THE INVENTION

Serogroup B and C strains of *Neisseria meningitidis* (Nm) together account
15 for the majority of invasive diseases in Europe and the United States. Vaccines against
individual Nm serogroups are presently available. The NIPH (National Institute of Public
Health of Norway) NmB vaccine is safe, elicits strain-specific immunity in children and
adults, and is efficacious in preventing NmB disease in adolescents. This vaccine has been
typically combined with meningococcal C polysaccharide vaccine and given with alum. The
20 plain polysaccharide vaccine component, however, is not effective in infants and young
children. The Chiron NmC conjugate (conj.) vaccine is also safe, elicits high titers of serum
bactericidal antibody in infants vaccinated as young as two and three months of age, and

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induces immunologic B cell memory to the unconjugated NmC polysaccharide. Since both serogroups cause disease, a combination vaccine which induces an immune response to both serogroups would be highly advantageous.

SUMMARY OF THE INVENTION

5 In one aspect, the present invention relates to an immunogenic composition or vaccine comprising NmC oligosaccharides conjugated to a carrier protein, NmB outer membrane proteins, and a carrier. In a preferred embodiment, the carrier protein is CRM₁₉₇, a non-toxic diphtheria toxin, the NmB outer membrane proteins are presented as proteoliposomic vesicles, and the carrier is aluminum hydroxide or MF59.

10 In another aspect, the present invention relates to a method of inducing an immune response to NmB and NmC, or vaccinating, comprising the administration of an immunologically effective amount of an immunogenic composition comprising NmC oligosaccharides conjugated to a carrier protein, NmB outer membrane proteins, and a carrier.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B summarize NmB IgG and NmC IgG antibody titers, respectively, as determined by ELISA.

Figures 2A and 2B summarize of titers of serum bactericidal antibody to NmB and NmC, respectively.

20 Figure 3 summarizes the comparison of antibody ratios to NmB and NmC induced by the combination vaccine in MF59 adjuvant vs. Alum.

Figure 4 summarizes the comparison of antibody ratios to NmB and NmC induced by the combination vaccine vs. the respective monovalent vaccine.

25 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A combination vaccine for NmB and NmC which induces an immune response to both serogroups that is not significantly different from the immune response induced by each serogroup alone is described. The immunogenicity of the NIPH NmB vaccine (referred to herein as "NmB" or "MenB" vaccines) and the Chiron NmC conjugate vaccine (referred to herein as "NmC conj." or "MenC conj."), alone, in combination, and in combination with

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the adjuvant MF59 is described herein.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of immunology and microbiology. Such techniques are explained fully in the literature. See, e.g., *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.) and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., Blackwell Scientific Publications).

As used herein, the term "immunogenic" refers to material which induces the production of antibody upon administration to a vertebrate, including humans.

As used herein, the term "carrier" refers to a pharmaceutically acceptable component other than the NmB or NmC immunogenic component. The carrier can be organic, inorganic, or both. Suitable carriers well known to those of skill in the art and include, without limitation, large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes) and inactive virus particles. The carrier can also function as an immunostimulatory agent, e.g., adjuvant. Suitable adjuvants are well known to those of skill in the art.

As used herein, the term "immunologically effective amount," means the administration of that amount, either in a single dose or as part of a series, that is effective for inducing the production of antibody for either the treatment or prevention of disease. This amount will vary depending upon a variety of factors, including the physical condition of the subject, and can be readily determined by someone of skill in the art.

As used herein, the term "vaccine" means an immunogenic composition which is able to induce a microbicidal immune response. Preferably, the vaccines of the present invention elicit a bactericidal antibody response.

The present invention is directed, in part, to immunogenic compositions which induce an immune response to both Meningitidis B and C. In preferred embodiments of the invention, the immunogenic composition comprises NmB outer membrane protein, and NmC oligosaccharide conjugated to a first carrier.

The NmB protein preferably comprises partially purified outer membrane proteins from strain 44/76 (B15:P1.7, 16:L3,7,9). The partially purified outer membrane proteins are preferably present as proteoliposomic vesicles as a result of the extraction process

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using deoxycholate. The dosage of NmB is expressed in μg of protein. Preferably, the NmB immune composition/vaccine components can be obtained from the National Institute of Public Health of Norway (NIPH). The NmB/alum vaccine comprises 0.05 mg/ml NmB protein, 3.33 mg/ml $\text{Al}(\text{OH})_3$ (alum), and 0.10 mg/ml thiomersalsodium.

The Chiron oligosaccharide represents NmC polysaccharide fragments of from preferably about 12 to about 22 repeating units. Preferably, the NmC oligosaccharide is conjugated to a first carrier. The dosage of NmC conjugate or polysaccharide is expressed in μg of sialic acid. An NmC vaccine containing unconjugated polysaccharide (referred to herein as "NmC polysaccharide" or "MenC Ps") can also be used. MenC Ps is a crude isolate comprising polysaccharides preferably from about 60 to about 80 repeating units.

In preferred embodiments of the invention, the first carrier is a protein, polysaccharide, polylactic acid, polyglycolic acid, polymeric amino acids, amino acid copolymer, lipid aggregate, or inactive virus particle. More preferably, the first carrier is a protein. Most preferably, the first carrier is CRM₁₉₇. Ten μg of oligosaccharide to 12.5-33 μg CRM₁₉₇ (i.e., to maintain a oligo/protein ratio of from about 0.3 to about 0.8) is preferably used per dose. More preferably, about 20 μg of CRM₁₉₇ can be used.

In preferred embodiments of the invention, the immunogenic composition comprises a second carrier, preferably, aluminum hydroxide (alum) or MF59. Alum can be obtained from Superfos, Bedbaek, Denmark, and is a 3% solution. When present, about 1 mg to about 1.67 mg of alum is used per dose. MF59 is a micro-fluidized emulsion of squalene in water that has been shown to be safe and to augment serum antibody responses to a variety of investigational vaccines. MF59 comprises about 5% squalene, 0.5% Tween 80 and about 0.5% Span 85. The adjuvant MF59 is described in PCT publication No. WO 90/14837, incorporated herein by reference in its entirety. MF59 can be made according to the procedures described in, for example, Ott *et al.*, *Vaccine Design: The Subunit And Adjuvant Approach*, 1995, M.F. Powell and M.J. Newman, Eds., Plenum Press, New York, p. 277-296; Singh *et al.*, *Vaccine*, 1998, 16, 1822-1827; Ott *et al.*, *Vaccine*, 1995, 13, 1557-1562; and Valensi *et al.*, *J. Immunol.*, 1994, 153, 4029-39, the disclosures of which are incorporated herein by reference in their entirety.

The immunogenic composition of the invention will employ an immunologically effective amount of the antigens. That is, there will be included an amount

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of antigen which, in combination with the adjuvant, will cause the subject to produce a specific and sufficient immunological response, preferably a T or B lymphocyte response, so as to impart protection to the subject from the subsequent exposure to *Neisseria*.

No single dose designation can be assigned which will provide specific guidance for each and every antigen which can be employed in this invention. The effective
5 amount of antigen will be a function of its inherent activity and purity and is empirically determined by those of ordinary skill in the art via routine experimentation.

The immunogenic compositions according to the present invention comprise an immunostimulatory amount of *Neisseria* antigen. An immunostimulatory amount is that amount which is sufficient to induce a measurable humoral or cellular immune response. For
10 example, the immunogenic compositions of the present invention comprise about 1 nanogram to about 1000 micrograms of antigen or about 10 nanograms to about 800 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 0.1 to about 500 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 1 to about 350 micrograms of antigen. In some preferred
15 embodiments, the immunogenic compositions contain about 25 to about 250 micrograms of antigen. In some preferred embodiments, the immunogenic compositions contain about 100 micrograms of antigen. One skilled in the art can readily formulate an immunogenic composition comprising any desired amount of antigen, which can be empirically determined by those of ordinary skill in the art via routine experimentation. The immunogenic
20 compositions can be conveniently administered in unit dosage form and can be prepared by any of the methods well known in the pharmaceutical art, for example, as described in Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, PA, 1980), the disclosure of which is incorporated herein by reference in its entirety.

The present invention is also directed to vaccines comprising any of the
25 immunogenic compositions described above.

The present invention is also directed to methods of inducing an immunologic response to NmB and NmC comprising administering an immunologically effective amount of an immunogenic composition described above to a human. Administration can be by any mode known to those skilled in the art including by oral, parenteral, pulmonary, transdermal,
30 rectal, intraperitoneal, intramuscular, or subcutaneous routes.

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The invention is further illustrated by way of the following examples which are intended to elucidate the invention. The foregoing examples are meant to illustrate the invention and are not to be construed to limit the invention in any way. Those skilled in the art will recognize modifications that are within the spirit and scope of the invention. All references cited herein are hereby incorporated by reference in their entirety.

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EXAMPLES

Example 1: ELISA results

Groups of guinea pigs (n=15 animals) were assigned to receive one of the following vaccines set forth in Table 1:

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Table 1

<u>Group</u>	<u>Components</u>	<u>Amount per dose</u>
Group 1	NmC conj./alum	10 µg /1 mg
Group 2	NmB/alum	25 µg/1 mg
Group 3	NmC polysaccharide/NmB/alum	10 µg /25 µg /1 mg
15 Group 4	NmC conj./NmB/alum	10 µg/25 µg/1 mg
Group 5	NmC conj./NmB/MF59	10 µg /25 µg /0.5 ml.

Group 6 (n=5) comprised control animals that received alum alone.

Eighty guinea pigs were randomized into the groups set forth above and received one of six vaccine combinations. For the data presented in Table 2, each animal received two injections, IM, separated by 28 days. Serum samples were obtained prior to each injection, and 18 days after the second injection. For the data presented in Figures 1A and 1B, each animal received two immunizations separated by six weeks. Each dose consisted of two 0.25 ml IM injections. Serum samples were obtained immediately prior to each injection, and 14 or 18 days after the second injection.

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Serum samples were assayed for IgG anticapsular antibody concentrations to NmC (Table 2 and Figure 1A) and for IgG anti-outer membrane vesicle antibody concentrations to NmB by ELISA (Figure 1B). The ELISA data were generated in a representative assay of individual animal sera (Table 2) and also expressed as averages from a plurality of assays (Figures 1A and 1B). The summary ELISA data set forth in Table 2 are, therefore, expressed as geometric means.

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For the ELISA, MCPS-ADH (NmC polysaccharide-adipic acid dihydrazide) conjugate or outer membrane vesicle (OMV) components was coated onto polystyrene microtiter plates overnight at 4°C, 1 µg/ml, 100 µl/well. On each coated plate, 100 µl/well of each of a reference standard (i.e., pooled guinea pig serum), a positive control, a negative control, and the serum samples were two-fold serially diluted in a buffer containing 75 µM ammonium thiocyanate, and incubated for two hours at room temperature. Rabbit anti-guinea pig IgG antibody conjugated to peroxidase was added to the wells (100 µl/well). After 2 hours, the colorimetric substrate 3,3',5,5', Tetramethylbenzidine (TMB) (100 µl/well) was added, and the color was developed for 15 minutes. The levels of antibodies to MCPS ant to OMV present in the controls and samples were obtained from a standard curve using the reference standard which has an assigned value of 100 ELISA units/ml. The results are shown in Table 2 and Figures 1A and 1B.

The results summarized in Table 2 and Figures 1A and 1B reveal that the combination vaccine was immunogenic, as measured by NmB and NmC IgG antibody titers, respectively. Figure 1A shows that a specific anti-meningococcal B antibody response was induced by the vaccine combinations comprising NmB. Figure 1B shows that a specific anti-meningococcal C antibody response was induced by the vaccine combinations comprising NmC. In particular, the antibody response induced by the combination of the NmC conjugate and NmB in the presence of MF59 adjuvant (Group 5) was significantly greater than the antibody response induced by either the NmC conjugate alone (Group 1) or the combination of the NmC conjugate and NmB in the presence of alum (Group 4). When the adjuvant MF59 was present, the antibody titer for the combination vaccine increased approximately six-fold.

Table 2: IgG MenC Antibody Responses (GMT)

Vaccine	Adjuvant	SCN Assay	
		Post-1	Post-2
MenC Conj.	Alum	20.3	155
MenB	Alum	<1	<1
MenC Ps + MenB	Alum	<1	1.5

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MenC Conj. + MenB	Alum	9.5	71
MenC Conj. + MenB	MF59	15.2	426
none	Alum	<1	<1

5

Example 2: Bactericidal Titers

Serum samples were tested for complement-mediated bactericidal titers to MenC strain 60E and MenB strain 44/76. Bactericidal titers were assayed on pooled sera from each group. Bactericidal data were generated using human complement.

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Components of the assay (i.e., buffer, antibody, complement, and bacteria) were added to sterile, 96-well tissue culture plates with lids (Nunc # 167008). The plates were maintained at room temperature during the assay. To each well, 50 μ l Gey's buffer (Gibco) containing 1% RIA Grade BSA (Sigma), 25 μ l of the diluted test antibody, 25 μ l of bacteria diluted 1:8000 in Gey's buffer/1% BSA, were sequentially added. Control wells include 1) Gey's buffer/1% BSA and bacteria alone (to determine if the organisms are viable in the diluent alone); 2) a time 0 control containing 75 μ l buffer, 25 μ l heat-inactivated (56°C, 30 min.) human complement, and 25 μ l bacteria; and 3) a toxicity control testing the complement at 20% and 40% with buffer and bacteria to verify that the complement source is non-toxic to the test strain. All antibody samples (at the highest concentration assayed) were also tested with heat-inactivated complement to show that a decrease in colony forming units (cfu) in the presence of antibody is complement dependent. After all reagents were added, 22 μ l was taken from each control well and plated onto Mueller-Hinton agar plates by allowing the sample to run from the top to the bottom of the plate, to determine the cfu in the well at 0 min. The microtiter plates were then covered and sealed with parafilm, and rotated gently for 1 hour at 37°C in a 4% CO₂ incubator. The plates were then removed, and a 22 μ l sample from each well plated on Mueller-Hinton agar. The culture plates were incubated for about 18 hours at 37°C, with 4% CO₂. The colonies were counted, and % survival determined for each test well: % survival = ([cfu of sample well at 60 min]/[cfu in the heat inactivated complement control well at time 0 min.]) x 100. Bactericidal titers reported are those which resulted in 50% survival. Results from a single experiment are presented in Table 3. Results are also

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presented in Figures 2A and 2B, with Figure 2B representing average titers from a plurality of experiments.

As the results summarized in Table 3 reveal, the combination vaccine elicited high titers of serum bactericidal antibody for both NmB and NmC. Bactericidal NmC antibody titer was slightly higher for the combination vaccine using MF59 as the carrier, but there was essentially no effect on bactericidal NmB titer using MF59. Interestingly, two- to five-fold higher NmB bactericidal titers were obtained with the combination vaccine than with the NmB vaccine alone. Figure 2A demonstrates that the antibodies directed to meningococcal B induced by the vaccine combinations comprising NmB were bactericidal. Figure 2B demonstrates that the antibodies directed to meningococcal C induced by the vaccine combinations comprising NmC conjugate were also bactericidal.

Table 3

Group Vaccine	NmC (1/titer)			NmB (1/titer)		
	Pre	Post-1	Post-2	Pre	Post-1	Post-2
NmC conj. + Alum	<5	80	>3375	<5	<5	<5
NmB + Alum	<5	<5	15	<5	15	800
NmC Ps + NmB + Alum	<5	<5	30	<5	25	1500
NmC Conj. + NmB + Alum	<5	25	2000	<5	25	5000
NmC Conj. + NmB + MF59	<5	50	>3375	<5	25	4000
Alum	<5	<5	<5	<5	<5	<5

Example 3: Comparison of Alum and MF59 Adjuvants

Serum from the animals described above in Figures 1A and 1B were compared and MenC and MenB antibody responses generated by NmB/NmC conj. in either alum or MF59 adjuvant were detected as described above in Examples 1 and 2. The results, shown in Figure 3, demonstrate that the antibody response to meningococcal C was approximately

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6-fold greater in vaccines comprising MF59 adjuvant.

Example 4: Comparison of Antibody Responses Generated by Combination Vaccine to Monovalent Vaccines

5 Serum from the animals described above in Figures 1A and 1B were compared
and MenC and MenB antibody responses generated by NmB/NmC conj. were compared with
the antibody responses generated by either the NmB vaccine alone or the NmC conj. alone in
alum as described above in Examples 1 and 2. The results, shown in Figure 4, demonstrate
that there is no significant difference in the antibody responses to the components of the
10 NmB/NmC conj. vaccine compared to the responses induced by the respective monovalent
vaccines (either NmB or NmC conj.).

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WHAT IS CLAIMED IS:

1. An immunogenic composition comprising NmC oligosaccharide conjugated to a first carrier and NmB outer membrane protein.
2. The immunogenic composition of claim 1 wherein said first carrier is selected from the group consisting of protein, polysaccharide, polylactic acid, polyglycolic acid, polymeric amino acids, amino acid co-polymer, lipid aggregate, and inactive virus particle.
3. The immunogenic composition of claim 2 wherein said first carrier is a protein.
4. The immunogenic composition of claim 3 wherein said first carrier is CRM₁₉₇.
5. The immunogenic composition of claim 1 the NmB outer membrane protein is presented as proteoliposomic vesicles.
6. The immunogenic composition of claim 1 wherein said composition comprises a second carrier.
7. The immunogenic composition of claim 6 wherein said second carrier is aluminum hydroxide or MF59.
8. A method of inducing an immunologic response to NmB and NmC comprising administering an immunologically effective amount of an immunogenic composition of claim 1.
9. The method of claim 8 wherein said first carrier is selected from the group consisting of protein, polysaccharide, polylactic acid, polyglycolic acid, polymeric amino acids, amino acid co-polymer, lipid aggregate, and inactive virus particle.
10. The method of claim 9 wherein said first carrier is a protein.

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11. The method of claim 10 wherein said first carrier is CRM₁₉₇.
12. The method of claim 8 the NmB outer membrane protein is presented as proteoliposomic vesicles.
- 5 13. The method of claim 8 wherein said composition comprises a second carrier.
14. The method of claim 13 wherein said second carrier is aluminum hydroxide or MF59.
- 10 15. A vaccine comprising an immunogenic composition of any one of claims 1-7.
16. A method of vaccinating an individual comprising administering to said individual an immunogenic composition of any one of claims 1-7.

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Figure 1A

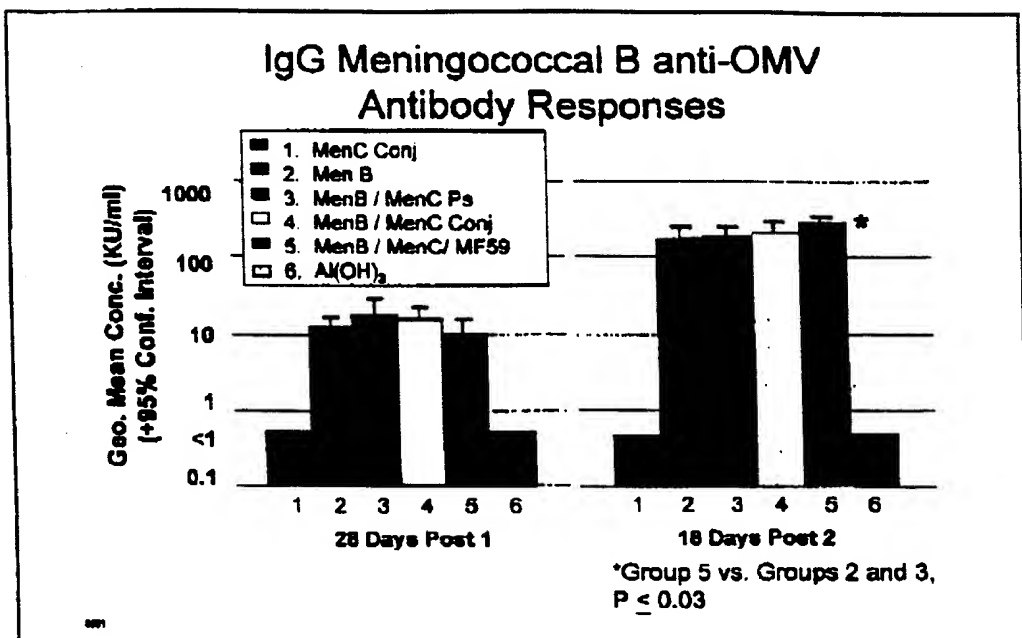
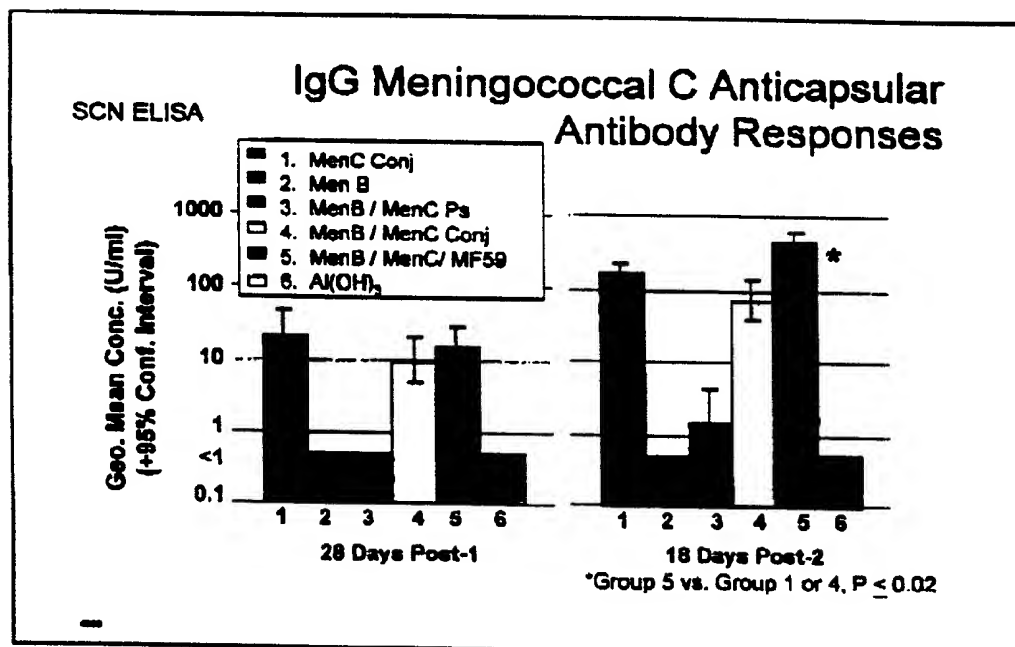


Figure 1B



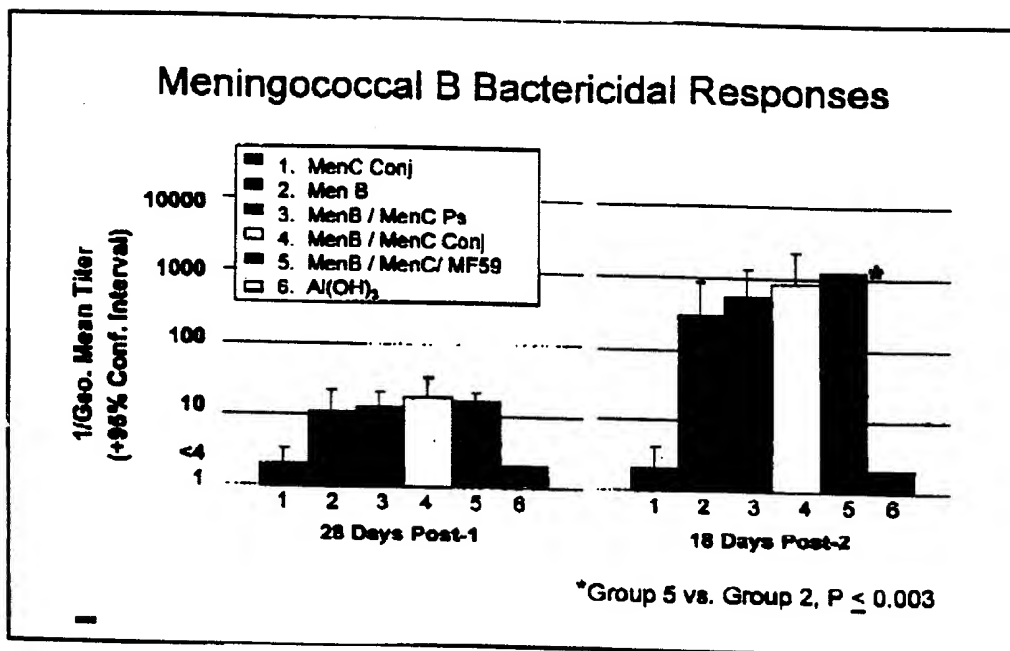
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Figure 2A

Figure 2B

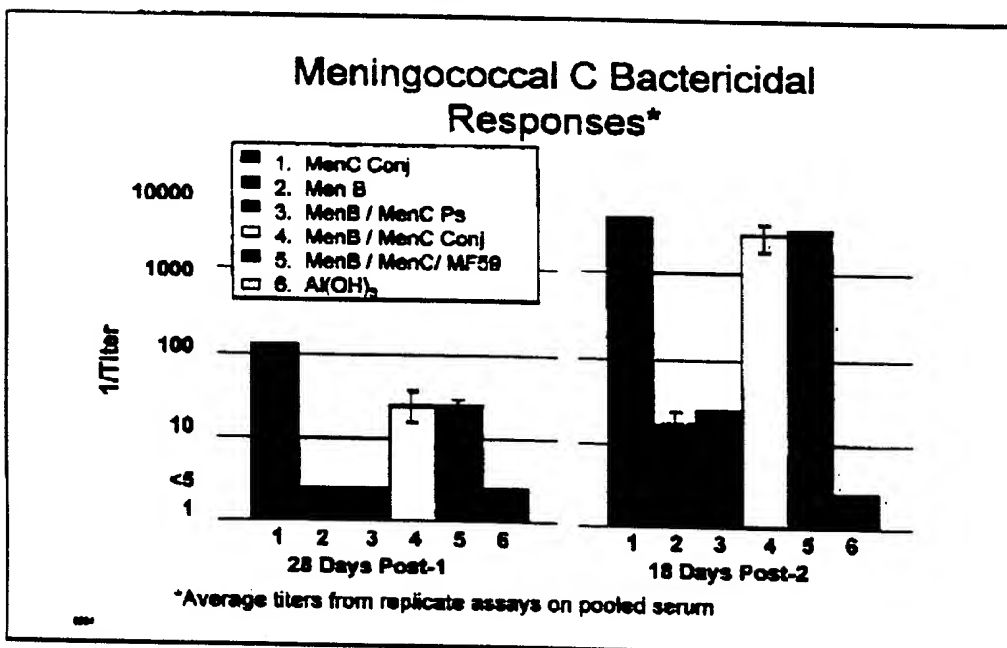


Figure 3

Ratios of Antibody Responses of Animals given Combination MenB OMV / MenC Conjugate with MF59 or Al(OH) ₃			
Assay		Ratio of GMT MF59 : GMT Al(OH) ₃	
		28 days Post - 1	18 days Post - 2
MenC	IgG	1.6	6.0**
	Bactericidal	1.0*	1.2*
MenB	IgG	0.7	1.4
	Bactericidal	0.9	1.4
*Pooled sera only tested		**P ≤ 0.001	

Figure 4

Ratios of Antibody Responses of Animals given Combination / Al(OH) ₃ vs. Monovalent / Al(OH) ₃			
Assay		Ratio of GMT Combo : GMT Mono	
		28 days Post - 1	18 days Post - 2
MenC	IgG	0.5	0.5
	Bactericidal	0.2*	0.7*
MenB	IgG	1.3	1.2
	Bactericidal	1.6	2.9**
*Pooled sera only tested.			
		**P ≤ 0.05	

INTERNATIONAL SEARCH REPORT

International Classification No

PCT/US 99/11977

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K39/095 A61K39/39 A61K9/127 A61K39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>WO 90 06696 A (RIJKINSINSTITUUT VOOR VOLKSGEZ ;PRAXIS BIOLOG INC (US)) 28 June 1990 (1990-06-28) page 4, line 6-20 page 9, line 1 - page 10, line 7 examples 2,7,10,15 claims</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-16
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

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PCT/US 99/11977

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIEBERMAN J M ET AL: "Safety and immunogenicity of a serogroups A/C <i>Neisseria meningitidis</i> oligosaccharide-protein conjugate vaccine in young children. A randomized controlled trial." JAMA, (1996 MAY 15) 275 (19) 1499-503. , XP002111434 page 1500, column 2, paragraph 2 page 1503, column 1, paragraph 3 - column 2 see reference 33 tables 2,3 figure 2	1-4, 6-11, 13-16
A	MILAGRES L G ET AL: "Antibody studies in mice of outer membrane antigens for use in an improved meningococcal B and C vaccine." FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, (1996 JAN) 13 (1) 9-17. , XP002111435 abstract page 9, left-hand column, paragraph 1 page 10, left-hand column, paragraph 5 page 15, right-hand column, paragraph 2 - page 16, right-hand column figure 5	1-19
A	DEBBAG R ET AL.: "Evaluation of adverse reactions associated to antimeningococcal BC vaccination in 16.700 children" CLIN.INFECT.DIS., vol. 21, September 1995 (1995-09), page 790-A420 XP002111436 the whole document	1-19
A	GRANOFF D M ET AL: "MF59 adjuvant enhances antibody responses of infant baboons immunized with <i>Haemophilus influenzae</i> type b and <i>Neisseria meningitidis</i> group C oligosaccharide - CRM197 conjugate vaccine." INFECTION AND IMMUNITY, (1997 MAY) 65 (5) 1710-5. , XP002081016 abstract table 1 page 1711, left-hand column, paragraphs 3,4 page 1713, right-hand column, paragraph 2 - page 1714, left-hand column, paragraph 1 page 1714, right-hand column, paragraph 2	1-19

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INTERNATIONAL SEARCH REPORT

International . . . cation No

PCT/US 99/11977

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	<p>ROSENQVIST E ET AL: "Effect of aluminium hydroxide and meningococcal serogroup C capsular polysaccharide on the immunogenicity and reactogenicity of a group B Neisseria meningitidis outer membrane vesicle vaccine."</p> <p>DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION, (1998) 92 323-33. , XP002111437</p> <p>abstract</p> <p>tables 1,2</p> <p>page 324, paragraph 2</p> <p>page 329, paragraph 2</p> <p>page 332, paragraph 2</p> <p style="text-align: center;">----</p>	1-19
T	<p>DATABASE MEDLINE 'Online!</p> <p>US national library of medicine (nlm)</p> <p>Bethesda , MD, US</p> <p>DIAZ ROMERO J ET AL.: "Current status of meningococcal group B vaccine candidates: capsular or noncapsular?"</p> <p>retrieved from STN</p> <p>Database accession no. 95136208</p> <p>XP002111438</p> <p>abstract</p> <p>& CLINICAL MICROBIOLOGY REVIEWS, vol. 7, no. 4, October 1994 (1994-10), pages 559-75,</p> <p style="text-align: center;">-----</p>	1-16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/11977

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8-14 16
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 8-14,16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 8-14 16

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Publication No

PCT/US 99/11977

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